GROWTH AND TOXICITY OF PRYMNESIUM PARVUM IN LONG-TERM CULTURES AT LOW TEMPERATURE AND SALINITY

by

XIANZI HE

Presented to the Faculty of the Graduate School of

The University of Texas at Arlington in Partial Fulfillment

of the Requirements

for the Degree of

MASTER OF SCIENCE IN EARTH AND ENVIRONMENTAL SCIENCE

THE UNIVERSITY OF TEXAS AT ARLINGTON

December 2010

ACKNOWLEDGEMENTS

First of all I would like to thank my supervisor Dr. James P. Grover for abundant help and his prolific suggestions and helpful comments on the text. Also I thank my thesis committee members Dr. Andrew P. Kruzic and Dr. Kevin A. Schug for many helpful suggestions. I thank Betty Scarbrough and Zhichao (Ivy) Zheng for their advice and help in the lab. I would like to thank my friends who have been giving me constant inspiration and moral support. Particularly, I want to thank Mr. Ping Lu for his encouragement and support all the time. Finally I would like thank my parents who unremittingly supported me during my years of study.

December 22, 2010

ABSTRACT

GROWTH AND TOXICITY OF PRYMNESIUM PARVUM IN LONG-TERM CULTURES AT LOW TEMPERATURE AND SALINITY

Xianzi He, M.S.

The University of Texas at Arlington, 2010

Supervising Professor: James P. Grover

There is a paradox reported concerning observations of toxic blooms of *Prymnesium parvum* in winter in Texas (USA), and its growth performance in the laboratory. Culture experiments indicate relatively high optimum temperature and salinity for growth, conditions different from those of winter in Texas. Because previous laboratory experiments were of short duration, it was suggested that long-term culturing of many generations could allow physiological and epigenetic changes, as well as natural selection to take place, leading to improved growth performance at low temperature and salinity. In this study, the growth and toxicity of *P. parvum* was investigated under relatively low temperature and salinity, for two phases of culture. The experimental results show, in general, that *P. parvum* grows more rapidly at higher temperature and higher salinity during both phases of long-term repeated culturing. When the two phases of culturing are compared, *P. parvum* grows more slowly at 11℃ during Phase 2, while growth differs little at 20℃. Toxicity differs little between phases. These results show no evidence of acclimation or adaptation during culturing over the length of time examined.

These results support earlier short-term experiments indicating that this Texas strain of *P. parvum* displays slower growth at temperatures < 20 ℃ and salinities < 2 ppt than it does at higher temperatures and salinities.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

CHAPTER 1

INTRODUCTION

Harmful algal blooms (HABs) have become an important cause of water quality problems and now receive increasing attention form aquatic scientists. Harmful algal blooms have increased globally in fresh, estuarine, and costal marine waters (Van Dolah, 2000; Glibert *et al*., 2005; Granéli and Turner, 2006), perhaps due to ongoing eutrophication, aquaculture, and other anthropogenic changes (Baker *et al*., 2009). Most toxic algal blooms are those whose toxins accumulate in the food web.

Prymnesium parvum, golden alga, is one of the toxic algae associated with HABS and which has worldwide incidence (Baker *et at*., 2007). Confirmed *P. parvum* blooms in Texas have been documented since 1985 (Texas Parks & Wildlife 2003). Blooms have caused fish kills in 19 reservoirs along major river systems, including the Brazos, Rio Grande, Colorado, and Red rivers. *Prymnesium parvum* blooms in reservoirs have resulted in some 17.5 million fish killed and an estimated economic impact in the tens of millions of dollars as of 2003 (Texas Parks & Wildlife 2003). *P. parvum* release toxin not only killing fish, but also, primarily, killing their grazers to invade the habitat.

Previous studies of *P. parvum* at higher salinities suggest a relatively high optimum temperature for population growth, estimated to be 27 ℃ and a high optimal growth salinity of 22 ppt for a strain isolated in Texas (Baker et al. 2007), which was also used in this study. However, many observations in Texas have indicated that blooms of *P. parvum* occur in winter time, when the temperature is normally between 10 and 20 ℃. The paradox between lab experiments and field observation led to this investigation of *P. parvum* growth under conditions of low temperature and salinity. As a result, in this study, *P. parvum* was put in a stressful condition with relatively low salinity and temperature. As in previous experiments, stock cultures

were used as the first generation for incubation in the first phase of culture. However, during Phase 1 of growth in culture, acclimation or adaptation of the population from the standard stock cultures could occur. The first phase of culturing corresponds to several generations of growth allowing physiological and epigenetic changes, as well as natural selection to take place. It has been suggested that 5 to 20 generations (about 1 - 3 weeks) are required for such acclimation, and that accurate measures of growth under stressful conditions cannot be obtained without allowing such a period (Brand, 1982). It is therefore suggested that growth studies need to use long-term, repeated culturing to get accurate estimates of growth rate due to acclimation or adaptation. In this study, acclimation and adaptation were studied by introducing a second phase of culturing right after the first one. For this second phase, cultures were inoculated with cells that experienced the same stressful salinity and temperature during the first phase. Measurements of growth performance from the two phases were compared.

The primary hypothesis of this study is that growth performance of *P. parvum* will improve in Phase 2 cultures compared to Phase 1 cultures. The exponential growth rate will be higher, and so will the population density reached after 21 days of culture. A secondary hypothesis could be: *P. parvum* will also be more toxic in Phase 2 than in Phase 1. If both hypotheses are true, then the species' ability to grow and cause harmful blooms in winter conditions in Texas would be higher than indicated in short term experiments where no long-term acclimation or adaptation to stressful conditions was allowed.

CHAPTER 2

MATERIALS AND METHODS

This study investigated growth and toxicity of *Prymnesium parvum* under different temperatures and salinities, and two phases of growth in culture are compared. This experiment examined growth at two temperatures and three salinities, with cultures inoculated first from stock cultures grown under standard conditions, and then from the previous experimental culture grown under the same temperature and salinity. It consisted of a 2 X 3 factorial design, with temperatures of 11 and 20 ℃ combined with salinities of 0.5, 1.0 and 4.0 ppt. With quadruplicates of each treatment combination, a total of 24 cultures were prepared for each phase of culturing.

2.1 Stock Cultures

A strain of *P. parvum* from Texas inland waters was obtained from the University of Texas at Austin Culture Collection of Algae (UTEX LB 2797, Austin, Texas). Stock cultures were grown in a medium of Artificial Sea Water (ASW) based on Kester et al (1967), as modified by Baker et al. (2007), diluted to a working salinity of 5.8 g L^{-1} with ultrapure water (Millipore Milli-Q, 18 MΩ cm^{-1}). Stock cultures of *P. parvum* were grown in 75 ml cultures maintained by monthly transfers in an incubator at 20℃ and on a 12:12 h light: dark photoperiod with an irradiance of about 150 µE m^{-2} s^{-1}.

2.2 Preparation of Experimental Media

Experimental media were prepared identically for each phase of culturing in this experiment. For experimental cultures, the basal salts for ASW medium were diluted to working salinities by adding appropriate proportions to ultrapure water (Millipore Milli-Q). For each treatment, a 1-liter Erlenmeyer flask was filled with 800ml of medium at the designated salinity. In addition to quadruplicate flasks for each experimental treatment, a fifth flask was prepared and treated in

parallel, but without inoculation as a methodological blank for assays of toxicity. All the flasks were autoclaved and allowed to cool. Several nutrients were then added to standardized concentration to every salinity treatment aseptically by filtration through 0.2 µm nylon capsule filters using syringes -- nitrate, phosphate, trace metals, vitamins and bicarbonate – so that all cultures received the same nutrient concentrations as stock cultures of algae. By using these relatively high concentrations, the chance of growth limitation arising from the scarcity of inorganic nutrients was negated.

2.3 Inoculation of Culture Phases 1 and 2

For Phase 1 of culturing, each flask prepared as described above was inoculated with 100 cells mL^{-1} of *P. parvum* from the stock cultures; methodological blanks were not inoculated. Stock cultures used for inoculation were in the late exponential growth stage. For Phase 2, each experimental culture was inoculated from the corresponding culture in Phase 1. Inoculum sizes differed due to anticipated differences in population density achieved after 21 days of culturing in Phase 1: for cultures grown at the salinity of 4 ppt, 2 ml of culture from Phase 1 was used; for cultures grown at 0.5 and 1 ppt salinity, the volume was 20 ml.

Inoculated flasks of different salinities were distributed to incubators at different temperatures according to the experimental design. Flasks were mixed daily and rotated randomly to change their positions in the incubators.

2.4 Sampling and measurements

2.4.1 Cell concentration

Samples were taken on days 2, 3, 4, 5, 7, 9, 11, 14, 17, and 21. At each sampling, an aliquot of 5 ml was preserved with 0.15 ml of Lugol's iodine for later counting of *P. parvum*. Cell concentration of *P. parvum* was obtained by direct microscopic counts at 400X of 1 ml aliquots in a Sedgwick-Rafter cell.

2.4.2 Erythrocyte Lysis Assay

On day 21 of each phase, a 50 µl aliquot was sampled from each experimental culture and each methodological blank to test the toxicity of *P. parvum* using an Erythrocyte Lysis

Assay (ELA, Skingel et al. 2010). Sterile, 100% packed sheep erythrocytes were obtained from Innovative Research, Inc. and stored at 4℃ until use. A homogenized buffer medium (HBM) was prepared by diluting sterile RPMI 1640 culture medium (Sigma-Aldrich) by 10% with sterile distilled water and adding 0.005 mg / ml heparin sodium salt as anticoagulant. Prior to assay, microscopic cell counts using a hemacytometer were calibrated to determinations of optical density to obtain a suspension $1.00 \pm 0.05 \times 10^7$ blood cells/ml for use in further preparation of red blood cell suspensions (RBCS). For the determination of hemolytic activity, 330 µl of sheep erythrocyte suspension was combined with a 20 µl subsample of culture in a 1.5 ml microcentrifuge tube. This mixture was incubated in the dark for 1 h at room temperature. After incubation, the tubes were centrifuged at 2500 × *g* for 5 min and 200 µl of supernatant was transferred into 500 µl wells of 96-well plate. Absorbance of the supernatant at 414 nm was read by a plate reader (Synergy 2; BioTek Instruments, Inc., Winooski, Vermont) and converted to percent lysis using a linear seven-point calibration curve.

2.4.3 Particulate Phosphorous Analysis

On day 21 of each phase, a 50 ml aliquot was sampled from each experimental culture for particulate phosphorous (PP) analysis. Each sample was filtered through a 47 mm GF/F filter and frozen for later analysis. To determine PP, filters were put in Wheaton glass bottles and digested with 8 ml of freshly-prepared oxidation reagent (5% $K_2S_2O_8$). After autoclaving and cooling, the digestate was filtered again and pH was adjusted to 8.3. Then, Soluble Reactive Phosphorous was determined (Strickland *et al*, 1972)

2.4.4 Other Measurements

Chlorophyll *a* concentration was determined using fluorometric procedures, for aliquots of 10 ml sampled on day 2, day 5, day 9, day 17, and day 21. Aliquots were filtered through 25 mm GF/F filters, which were then frozen in 1 ml of a saturated $MgCO_3$ solution. Twenty-four hours prior to analysis, 9ml of acetone was added to each sample and all the samples were incubated in darkness. Acetone extracts were decanted from the filters and chlorophyll *a* concentration was determined with a fluorometer (10 – AU Turner Designs, Sunnyvale,

California) (Welschmever, 1994). Chlorophyll *a* concentrations of treatments in Phase 1 were too low to be detected given the sampled volume of 10 ml. Samples of 100 ml were filtered onto precombusted GF/F filters for determinations of particulate C and N with a Perkin-Elmer CHN analyzer. Like chlorophyll *a*, particulate N concentrations of many treatments in Phase 1 were too low to be detected. Therefore, only results for Phase 2 values of chlorophyll a and particulate N are presented. However, measurement of particulate C and P was sufficient for analysis for both phases. The pH of endpoint cultures was determined with a calibrated, temperature-compensated electrode (Orion Research Inc.). Conductivity were also measured on day 21 of each phase using an electrode (YSI 30, YSI Inc., Yellow Springs, Ohio, USA)

2.5 Statistic Analyses

All response variables of each phase were analyzed with a factorial ANOVA, to test for the main effects of temperature (2 levels) and salinity (3 levels), and for the interaction of temperature and salinity. To compare specific treatment means, Tukey's HSD was used (Kleinbaum et al. 1998). This ANOVA was followed by contrasts comparing each measurement of phase 1 with phase 2 in a paired t-test. In both ANOVA and contrast analyses, statistical significance was concluded when the null hypothesis of no effect could be rejected with a significance level of $P < 0.05$. Analyses were completed with Statistica 6.0 (StarSoft, Tulsa, Oklahoma, USA).

CHAPTER 3

EXPERIMENT RESULTS

Some cultures experienced exponential growth and reached late stationary phase by day 21 when cells were harvested (e.g., Figure 3.1). More cultures did not display a distinct exponential and stationary phase. For example, as shown in Figure 3.2, *P. parvum* had a relatively low end point concentration in such cultures (compare to Figure 3.1). By day 21 when cells were harvested, many of the cultures still had a trend to grow.

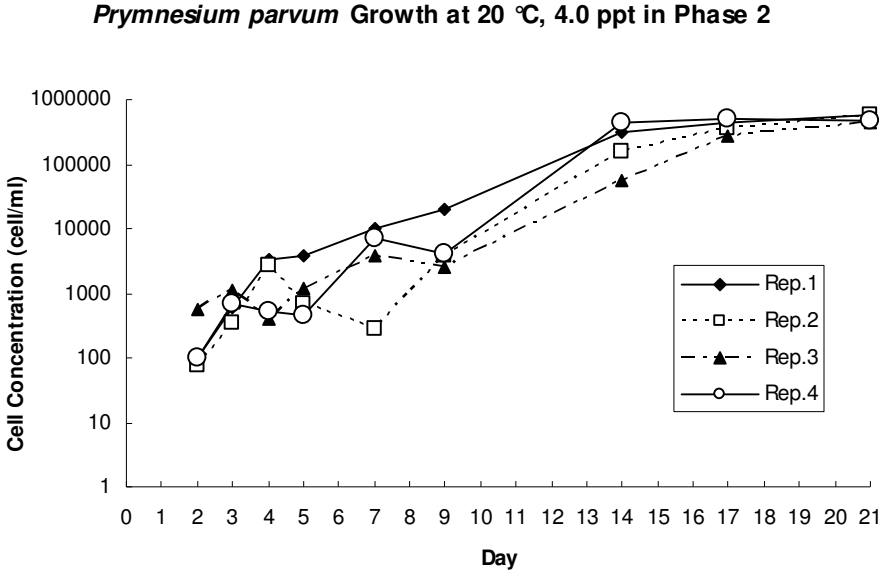


Figure 3.1 Example of a treatment where cultures displayed distinct exponential and stationary phases.

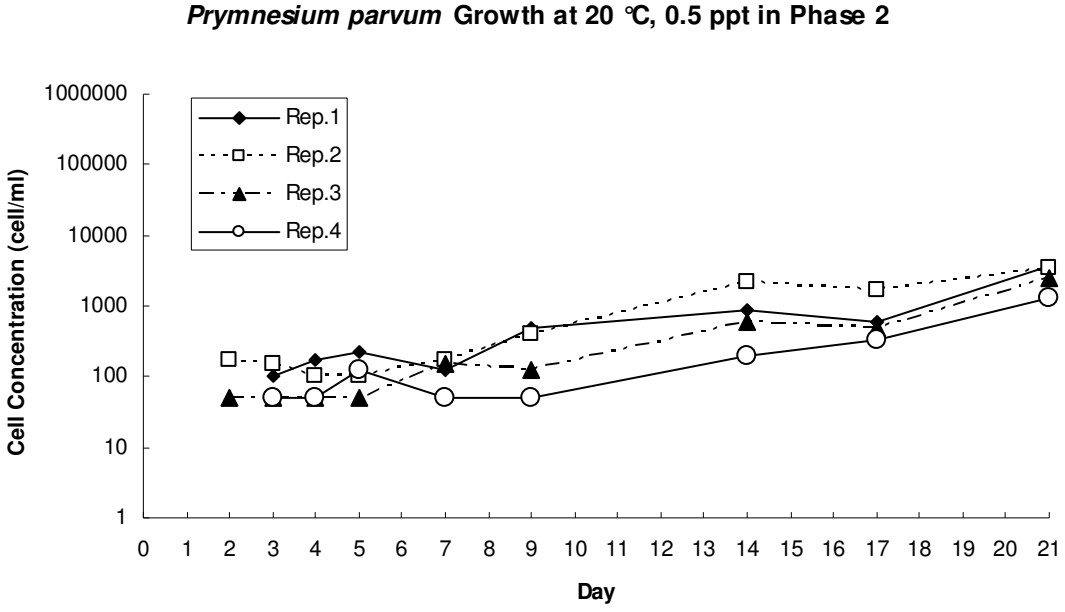


Figure 3.2 Example of a treatment where no stationary phase was evident.

All the measurements taken through the study are listed in Table 3.1, where undetectable values are coded to zero. Means of each primary measurement at each combination of treatments were analyzed by Statistica, using factorial ANOVA, with factors of temperature, salinity and their interaction.

Table 3.1 Experimental results of Phase 1 and Phase 2

Phase	Temperature (℃)	Salinity (ppt)	Replicate	Exponential Growth Rate (per day)	End Point Density (cell/ml)	Percent Lysis (%)	Particulate P (μM)	Chlorophyll α (μg/l)	pH	Conductivity (μs)	Particulate C (μmolC/ml)	Particulate N (μmolN/ml)
1	11	0.5	1	0.25	5.0×10^{2}	0	0.39	0	7.06	548	0.0449	-1.64×10^{-4}
1	11	0.5	2	0.16	5.0×10^{2}	0.6	0.47	0.03	7.24	547	0.0385	-3.36×10^{-4}
1	11	0.5	3	0.14	5.8×10^{2}	0.2	0.45	0	7.02	546	0.0369	5.00×10^{-4}
1	11	0.5	4	0.21	2.3×10^{3}	0.1	0.49	0.03	7.18	542	0.0458	1.64×10^{-4}
1	11	1	1	0.35	1.5×10^{5}	1.3	1.10	0	7.13	1019	0.0421	-1.64×10^{-4}
1	11	1	2	0.27	8.8×10^{3}	2.6	0.99	0	6.99	998	0.0379	-2.16×10^{-3}
1	11	1	3	0.18	2.0×10^{4}	3	1.07	0	6.86	992	0.0345	1.64×10^{-4}
1	11	1	4	0.22	3.5×10^{2}	1.4	1.04	0	7.10	926	0.0401	-3.36×10^{-4}
1	11	4	1	0.16	1.1×10^{3}	0.1	1.19	0.11	7.07	3337	0.0419	-1.83×10^{-3}
1	11	4	2	0.24	8.8×10^{4}	0	1.15	0.06	7.00	3433	0.0477	-6.64×10^{-4}
1	11	4	3	0.34	2.1×10^{5}	5.3	1.04	0.12	6.72	3388	0.0475	1.64×10^{-4}
1	11	4	4	0.13	1.1×10^{3}	2.9	1.17	0	6.96	3335	0.0542	-3.36×10^{-4}
1	20	0.5	1	0.26	1.0×10^{4}	2.2	0.47	0.37	6.81	531	0.0590	1.16×10^{-3}
1	20	0.5	2	0.26	2.1×10^{4}	2.9	0.4	0.1	6.86	532	0.0487	0
1	20	0.5	3	0.16	6.0×10^{3}	0.5	0.49	0	6.80	537	0.0723	8.29×10^{-4}
1	20	0.5	4	0.18	1.2×10^{3}	12.8	0.47	0	6.83	536	0.0463	-1.16×10^{-3}
1	20	1	1	0.32	1.9×10^{4}	18.3	0.77	0	7.10	984	0.0953	6.81×10^{-3}
1	20	1	2	0.32	3.4×10^{4}	16.9	1.02	0	6.88	988	0.0848	7.31×10^{-3}
1	20	1	3	0.21	3.2×10^{3}	0.4	1.04	1.81	7.07	988	0.0907	5.15×10^{-3}
1	20	1	4	0.23	1.2×10^{4}	4.1	0.49	0.01	7.10	994	0.0857	5.15×10^{-3}
1	20	4	1	0.46	3.4×10^{5}	75	1.74	0.73	7.52	3451	0.5799	6.91×10^{-2}
1	20	4	2	0.43	3.8×10^{5}	66.4	1.92	11.9	6.74	3420	0.6097	7.14×10^{-2}

Table 3.1 – *Continued*

1	20	4	3	0.35	2.1×10^{5}	45.9	1.76	1.23	7.73	3496	0.6197	7.74×10^{-2}
1	20	4	4	0.38	2.1×10^{5}	61.1	1.87	3.17	7.33	3450	0.6489	6.91×10^{-2}
2	11	0.5	1	0.03	50	17.8	0.74	0.03	6.94	490	0.0330	-2.66×10^{-3}
2	11	0.5	2	0.05	1.8×10^{2}	7	0.67	0	6.75	500	0.0326	3.82×10^{-3}
2	11	0.5	3	0.08	1.8×10^{2}	5.8	0.58	0	7.12	490	0.0270	3.36×10^{-4}
2	11	0.5	4	0.02	50	2.6	0.83	0.03	7.00	490	0.0283	1.64×10^{-4}
2	11	1	1	0.09	1.8×10^{2}	0	0.98	0	7.03	890	0.0318	4.49×10^{-3}
2	11	1	2	0.05	1.0×10^{2}	0	0.77	0	7.05	900	0.0472	3.66×10^{-3}
2	11	1	3	0.11	2.8×10^{2}	0	1.00	0.05	7.00	900	0.0323	2.16×10^{-3}
2	11	1	4	0.04	1.3×10^{2}	1	0.82	0	7.01	910	0.0307	3.36×10^{-4}
2	11	4	1	0.22	3.0×10^{3}	6.8	1.02	0.26	6.90	317	0.0418	6.64×10^{-4}
2	11	4	2	0.21	3.2×10^{3}	1.3	1.03	0.06	6.98	243	0.0432	3.99×10^{-3}
2	11	4	3	0.11	9.0×10^{2}	2.8	0.70	0.1	6.72	278	0.0339	4.49×10^{-3}
2	11	4	4	0.10	8.8×10^{2}	1.7	1.09	0.10	6.95	276	0.0765	4.49×10^{-3}
2	20	0.5	1	0.17	3.8×10^{3}	1.9	0.74	0.56	7.26	640	0.0361	2.99×10^{-3}
2	20	0.5	2	0.20	3.4×10^{3}	1.8	0.69	0.76	7.29	570	0.0398	1.83×10^{-3}
2	20	0.5	3	0.20	2.5×10^{3}	2.2	0.60	0.23	7.18	620	0.0304	2.16×10^{-3}
2	20	0.5	4	0.16	1.3×10^{3}	0.6	0.62	0.10	7.26	590	0.0307	8.29×10^{-4}
2	20	1	1	0.29	2.1×10^{4}	0	0.83	9.44	6.95	1100	0.1070	1.23×10^{-2}
2	20	1	2	0.38	3.4×10^{4}	0	0.89	2.74	7.34	1080	0.1052	1.10×10^{-2}
2	20	1	3	0.32	1.6×10^{4}	0	0.73	2.86	7.42	1090	0.1147	1.20×10^{-2}
2	20	1	4	0.33	7.4×10^{3}	9.2	0.89	1.36	7.32	1080	0.0903	7.14×10^{-3}
2	20	4	1	0.42	5.7×10^{5}	94	2.25	24.8	9.77	3600	0.5846	7.31×10^{-2}
2	20	4	2	0.47	5.6×10^{5}	75.7	1.72	25.9	9.63	2860	0.6673	8.87×10^{-2}
2	20	4	3	0.39	4.4×10^{5}	0	1.72	18.1	9.75	2860	0.5285	6.78×10^{-2}
2	20	4	4	0.48	4.7×10^{5}	6	1.42	24.1	9.37	3690	0.6017	5.45×10^{-2}

3.1 Phase 1 and Phase 2

3.1.1. Exponential Growth Rate

Exponential growth rate was calculated the slope of the natural logarithm of cell concentration versus time over the entire culturing interval of 21 days. Four high values of cell concentration were taken out as outliers, from four different cultures. In general, exponential growth rate increased with higher temperature and higher salinity (Figure 3.3). For both Phase 1 and Phase 2, the algae at 4.0 ppt and 20 ℃ had the highest growth rate and algae at 20 ℃ grew better than those at 11 ℃. Phase 2 growth at 20 degrees was similar to Phase 1, and Phase 2 growth at 11 degrees was lower than in Phase 1.

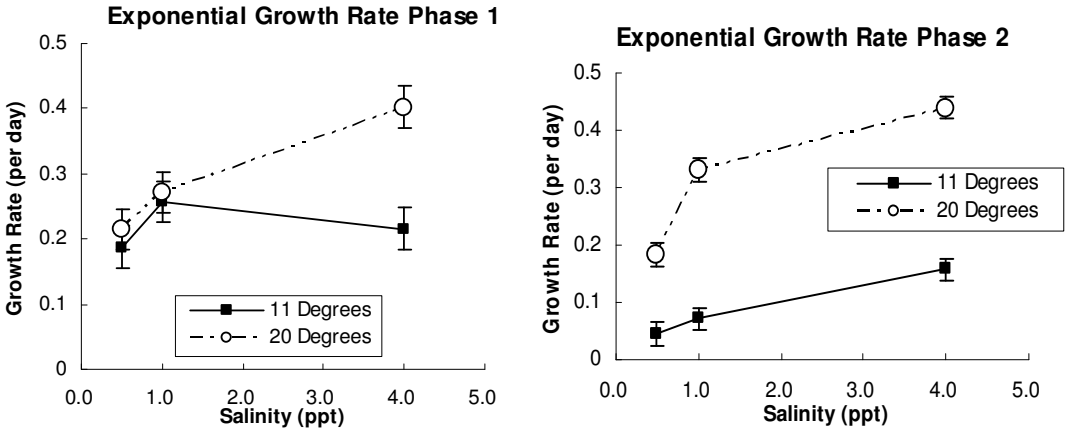


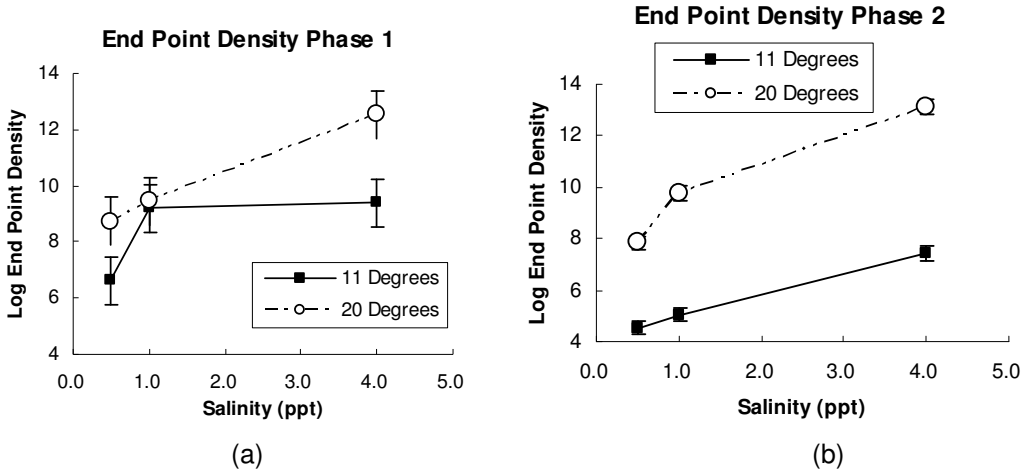
Figure 3.3 Exponential growth rates of (a) Phase 1 and (b) Phase 2. Error bars represent standard error based on the mean square error of the ANOVA.

In Phase 1, the experimental effect of temperature was statistically significant (ANOVA, $F_{(1,18)} = 8.48$, $P = 0.009$). The experimental effect of salinity was statistically significant (ANOVA, $F_{(2,18)} = 5.75$, $P = 0.012$). The interaction effect of temperature and salinity was statistically significant (ANOVA, $F_{(2,18)} = 4.54$, $P = 0.025$). The cultures at 20 ℃ and 4.0 ppt had significantly higher growth than all other treatments except for 20℃ and 4.0 ppt (Tukey's HSD test, $P < 0.05$). In Phase 2 the experimental effect of temperature was statistically significant (ANOVA, $F_{(1,18)} = 198.99$, $P < 0.001$). The experimental effect of salinity was statistically significant

(ANOVA, $F_{(2,18)}$ = 43.86, P < 0.001). The interaction effect of temperature and salinity was statistically significant (ANOVA, $F_{(2,18)}$ = 7.91, P = 0.003). The cultures at 20 ℃ and 4.0 ppt had significantly higher growth rate than all other treatments except for 20℃ and 4.0 ppt (Tukey's HSD test, P < 0.05). The cultures at 20 ℃ and 1.0 ppt also had significantly higher growth rate than all other treatments except for 20℃ and 4.0 ppt (Tukey's HSD test, P<0.05).

The main difference between Phase 1 and Phase 2 was the lower growth for the 11 ℃ cultures of Phase 2 than that of Phase 1.

3.1.2. End Point Density



(a) (b)

Figure 3.4 Log end point densities of (a) Phase 1 and (b) Phase 2. Error bars represent standard error based on the mean square error of the ANOVA.

End point densities showed a similar pattern as the exponential growth rate (Figure 3.4). In Phase 1, the experimental effect of temperature was statistically significant (ANOVA, $F_{(1,18)}$ = 6.92, P = 0.017). The experimental effect of salinity was statistically significant (ANOVA, $F_{(2,18)}$ = 7.33, P = 0.005). The interaction effect of temperature and salinity was not statistically significant (ANOVA, $F_{(2,18)}$ = 1.47, P = 0.26). The end point density was higher at 20 ℃ than at 11 ℃, and also increased with salinity.

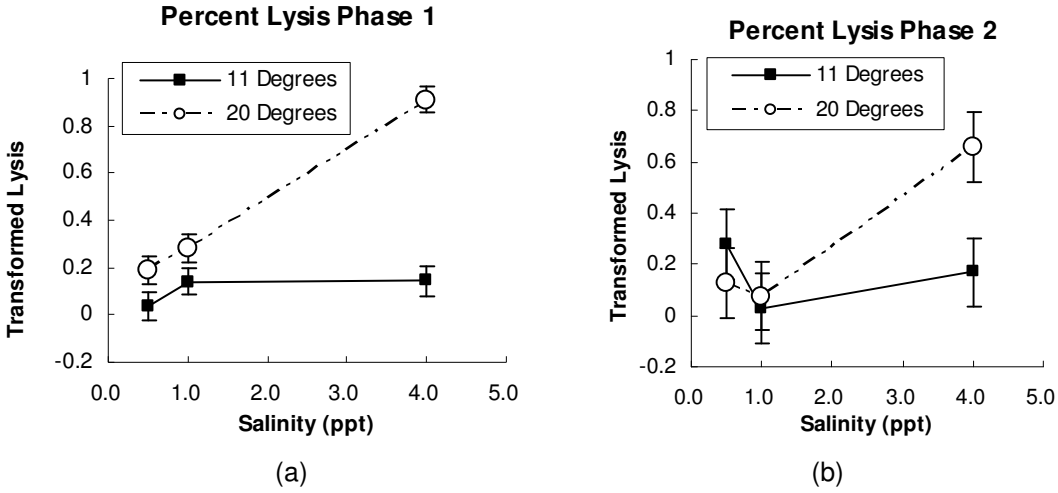
In Phase 2 the experimental effect of temperature was statistically significant (ANOVA, $F_{(1,18)}$ = 396.45, P < 0.001). The experimental effect of salinity was statistically significant

(ANOVA, $F_{(2,18)}$ = 111.62, P < 0.001). The interaction effect of temperature and salinity was statistically significant (ANOVA, $F_{(2,18)}$ = 9.44, P = 0.002). The cultures at 20 ℃ and 4.0 ppt and 1.0 ppt had significantly greater density than all the other cultures (Tukey's HSD test, P < 0.05).

The difference between Phase 1 and Phase 2 was the lower end point density at 11℃ of Phase 2 than that of Phase 1.

3.1.3. Percent Lysis

For statistical analysis, percent lysis obtained from ELA on day 21 was transformed by using the arcsin square root transformation, so that data would conform to the assumptions required for ANOVA. Figure 3.5 illustrated that for both phases, percent lysis was highest for cultures at 20 degrees, 4.0 ppt.



(a) (b)

Figure 3.5 Transformed percent lysis of (a) Phase 1 and (b) Phase 2. Error bars represent standard error based on the mean square error of the ANOVA.

In Phase 1, the experimental effect of temperature was statistically significant (ANOVA, $F_{(1,18)}$ = 53.90, P < 0.001). The experimental effect of salinity was statistically significant (ANOVA, $F_{(2,18)}$ = 25.70, P < 0.001). The interaction effect of temperature and salinity was statistically significant (ANOVA, $F_{(2,18)}$ = 17.56, P < 0.001). The cultures at 20 ℃ and 4.0 ppt had significantly higher overall toxicity than all the other cultures (Tukey's HSD test, P < 0.05).

In Phase 2 the experimental effect of temperature was not statistically significant (ANOVA, $F_{(1,18)} = 1.35$, $P = 0.26$). The experimental effect of salinity was not statistically significant (ANOVA, $F_{(2,18)} = 3.55$, $P = 0.05$). The interaction effect of temperature and salinity was not statistically significant (ANOVA, $F_{(1,18)} = 2.88$, $P = 0.08$).

The main difference between Phase 1 and Phase 2 was the higher toxicity for the 11 ℃ cultures of Phase 2 than that of Phase 1.

3.1.4. Percent Lysis per Cell

A measure of toxic activity per cell was obtained by dividing percent lysis by end point cell density. On this basis, cultures at 20℃ and 4.0 ppt did not show the greatest toxicity (Figure 3.6).

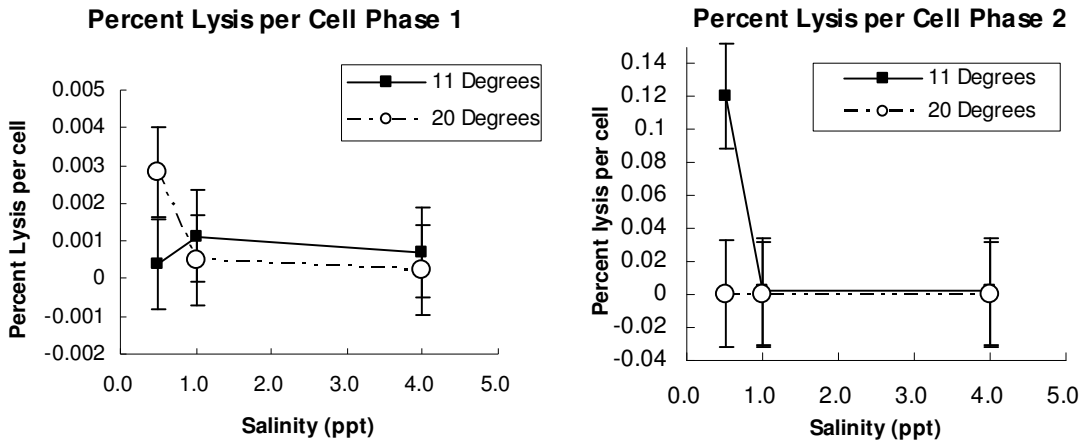


Figure 3.6 Percent lysis per cell of (a) Phase 1 and (b) Phase 2. Error bars represent standard error based on the mean square error of the ANOVA.

In Phase 1, the experimental effect of temperature was not statistically significant (ANOVA, $F_{(1,18)} = 0.20$, $P = 0.66$). The experimental effect of salinity was not statistically significant (ANOVA, $F_{(2,18)} = 0.47$, $P = 0.64$). The interaction effect of temperature and salinity was not statistically significant (ANOVA, $F_{(2,18)} = 1.04$, $P = 0.37$).

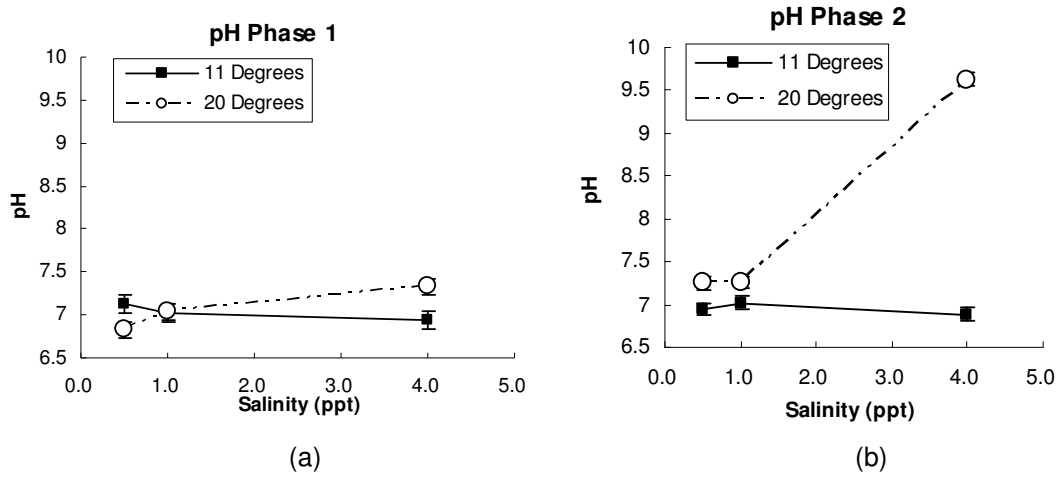
In Phase 2 the experimental effect of temperature was not statistically significant (ANOVA, $F_{(1,18)} = 2.46$, $P = 0.13$). The experimental effect of salinity was not statistically

significant (ANOVA, $F_{(2,18)}$ = 2.29, P = 0.13). The interaction effect of temperature and salinity was not statistically significant (ANOVA, $F_{(2,18)}$ = 2.26, P = 0.13).

The main difference between Phase 1 and Phase 2 was the higher toxicity for the 11 ℃ cultures of Phase 2 and higher toxicity of the 20℃ cultures of Phase 1.

3.1.5. pH Values

A pH measure was obtained at the last day of each phase (Figure 3.7). For most treatments, mean pH ranged 6.5 - 7.5, except for 20℃ and 4.0 ppt in Phase 2.



(a) (b)

Figure 3.7 pH values of (a) Phase 1 and (b) Phase 2. Error bars represent standard error based on the mean square error of the ANOVA.

In Phase 1, the experimental effect of temperature was not statistically significant (ANOVA, $F_{(1,18)}$ = 0.2, P = 0.66). The experimental effect of salinity was not statistically significant (ANOVA, $F_{(2,18)}$ = 1.29, P = 0.30). The interaction effect of temperature and salinity was statistically significant (ANOVA, $F_{(2,18)}$ = 5.96, P = 0.01). The cultures at 20℃ and 4.0 ppt had significantly higher pH than that the cultures at 20℃ and 1.0 ppt (Tukey's HSD test, P < 0.05). However, the difference in pH was not large, and all Phase 1 cultures had pH near 7.

In Phase 2 the experimental effect of temperature was statistically significant (ANOVA, $F_{(1,18)}$ = 363.46, P < 0.001). The experimental effect of salinity was statistically significant (ANOVA, $F_{(2,18)}$ = 176.21, P < 0.001). The interaction effect of temperature and salinity was statistically significant (ANOVA, $F_{(2,18)}$ = 208.41, P < 0.001). The cultures at 20℃ and 4.0 ppt

had significantly higher pH than all the other cultures (Tukey's HSD test, $P < 0.05$), by about 2 pH units.

The main difference between Phase 1 and Phase 2 is higher pH for the 20 ℃ cultures of Phase 2.

3.1.6. P Quota

P quota per cell was obtained by dividing particulate P by end point cell concentration. Figure 3.8 illustrates that *P. parvum* tended to have a decreasing P quota with increasing salinity and temperature.

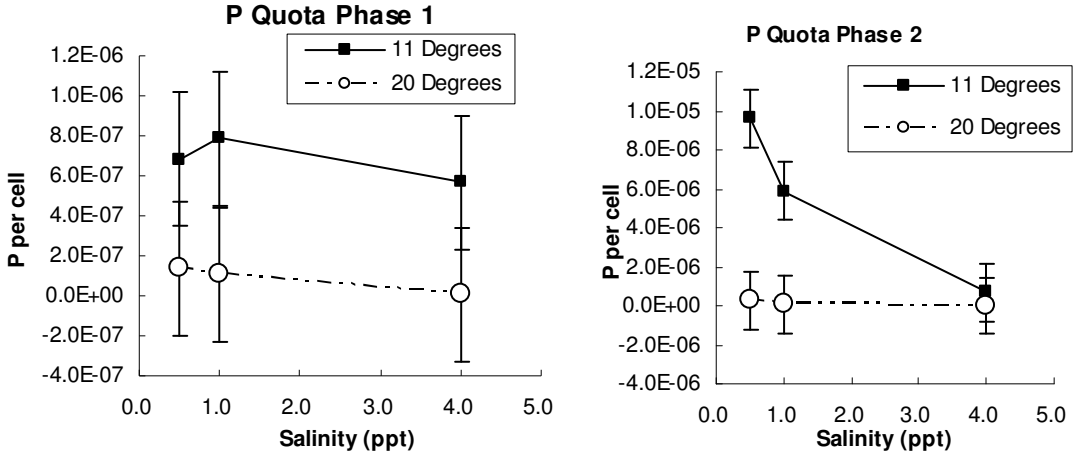


Figure 3.8 P quota of (a) Phase 1 and (b) Phase 2. Error bars represent standard error based on the mean square error of the ANOVA.

In Phase 1, the experimental effect of temperature was statistically significant (ANOVA, $F_{(1,18)} = 4.73$, $P = 0.04$). The experimental effect of salinity was not statistically significant (ANOVA, $F_{(2,18)} = 0.13$, $P = 0.88$). The interaction effect of temperature and salinity was not statistically significant (ANOVA, $F_{(2,18)} = 0.02$, $P = 0.98$).

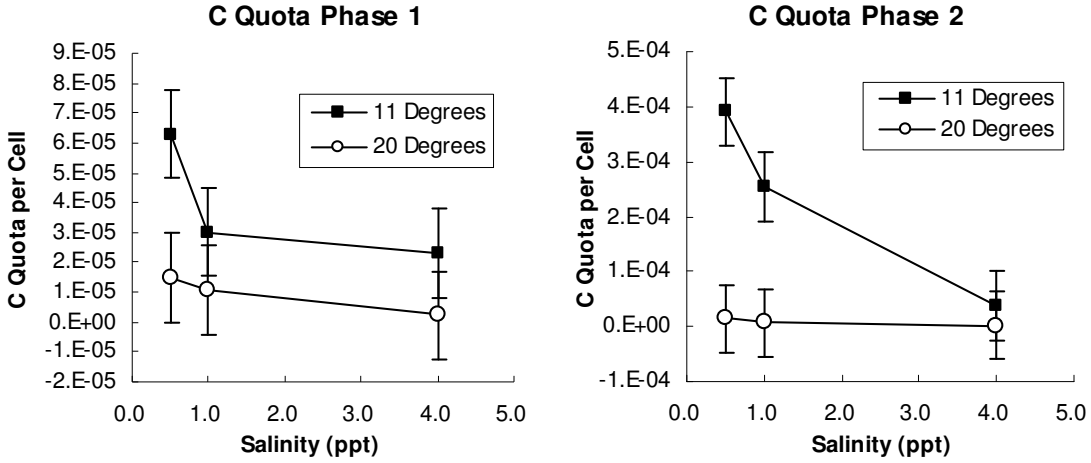
In Phase 2 the experimental effect of temperature was statistically significant (ANOVA, $F_{(1,18)} = 19.05$, $P < 0.001$). The experimental effect of salinity was statistically significant (ANOVA, $F_{(2,18)} = 4.88$, $P = 0.02$). The interaction effect of temperature and salinity was statistically significant (ANOVA, $F_{(2,18)} = 4.33$, $P = 0.03$). The cultures at 11℃ and 0.5 ppt had

significantly higher P quota than all the other cultures except for the cultures at 11°C and 1.0 ppt (Tukey's HSD test, $P < 0.05$).

The main difference between Phase 1 and Phase 2 was the higher P quota per cell for the 11 °C cultures of Phase 2.

3.1.7. C Quota

C quota per cell was obtained by dividing particulate C by end point cell concentration. Figure 3.9 illustrates that *P. parvum* had a decreasing C quota with increasing salinity and temperature.



(a) (b)

Figure 3.9 C quota of (a) Phase 1 and (b) Phase 2. Error bars represent standard error based on the mean square error of the ANOVA.

In Phase 1, the experimental effect of temperature was statistically significant (ANOVA, $F_{(1,18)} = 5.97$, $P = 0.03$). The experimental effect of salinity was not statistically significant (ANOVA, $F_{(2,18)} = 1.66$, $P = 0.22$). The interaction effect of temperature and salinity was not statistically significant (ANOVA, $F_{(2,18)} = 0.60$, $P = 0.56$).

In Phase 2 the experimental effect of temperature was statistically significant (ANOVA, $F_{(1,18)} = 19.06$, $P < 0.001$). The experimental effect of salinity was statistically significant (ANOVA, $F_{(2,18)} = 4.46$, $P = 0.03$). The interaction effect of temperature and salinity was statistically significant (ANOVA, $F_{(2,18)} = 3.85$, $P = 0.041$). The cultures at 11°C and 0.5 ppt had

significantly higher C Quota than all the other cultures except for the cultures at 11°C and 1.0 ppt (Tukey's HSD test, $P < 0.05$).

The main difference between Phase 1 and Phase 2 is lower C quota per cell for the 11 °C cultures of Phase 2.

3.1.8. N Quota Phase 2

N quota per cell was obtained by dividing particulate nitrate by end point cell concentration, for Phase 2 only because particulate N was undetectable for a large number of cultures in Phase 1. Figure 3.10 illustrates the *P. parvum* had a decreasing N quota with increasing temperature.

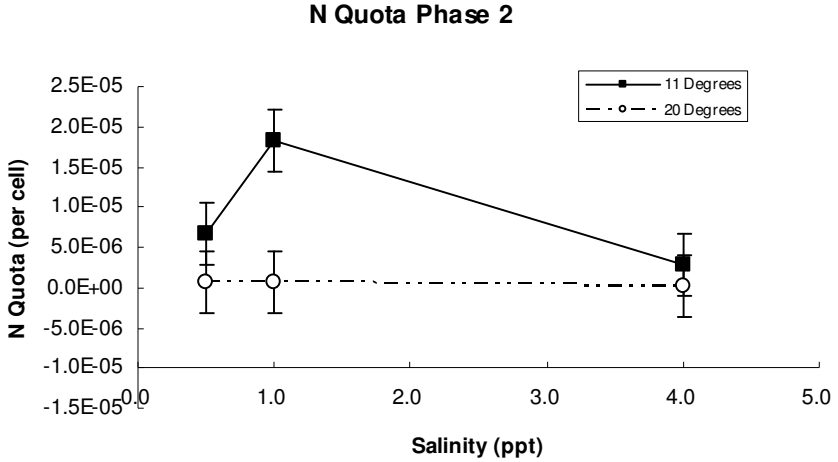


Figure 3.10 N quota of Phase 2. Error bars represent standard error based on the mean square error of the ANOVA.

In Phase 2, the experimental effect of temperature was statistically significant (ANOVA, $F_{(1,18)} = 7.79$, $P = 0.01$). The experimental effect of salinity was not statistically significant (ANOVA, $F_{(2,18)} = 2.24$, $P = 0.14$). The interaction effect of temperature and salinity was not statistically significant (ANOVA, $F_{(2,18)} = 2.03$, $P = 0.16$).

3.1.9. C:P Ratio

C:P ratio was obtained by dividing C quota by P quota. Figure 3.11 illustrated that *P. parvum* had an increasing C:P ratio with increasing salinity and temperature.

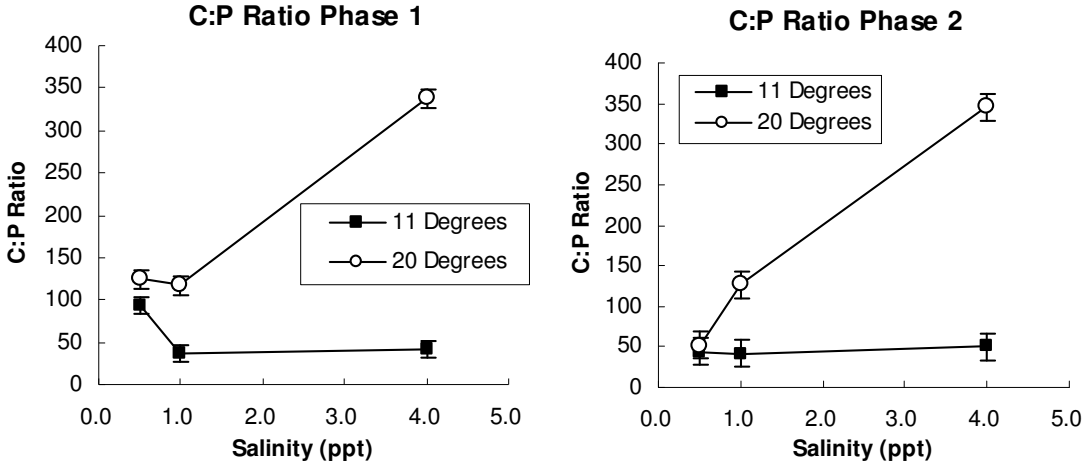


Figure 3.11 C:P ratio of (a) Phase 1 and (b) Phase 2. Error bars represented standard error based on the mean square error of the ANOVA.

In Phase 1, the experimental effect of temperature was statistically significant (ANOVA, $F_{(1,18)} = 245.61$, $P < 0.001$). The experimental effect of salinity was statistically significant (ANOVA, $F_{(2,18)} = 60.47$, $P < 0.001$). The interaction effect of temperature and salinity was statistically significant (ANOVA, $F_{(2,18)} = 87.92$, $P < 0.001$). The cultures at 20℃ and 4.0 ppt had significantly higher C:P ratio than all the other cultures (Tukey's HSD test, $P < 0.05$).

In Phase 2 the experimental effect of temperature was statistically significant (ANOVA, $F_{(1,18)} = 91.44$, $P < 0.001$). The experimental effect of salinity was statistically significant (ANOVA, $F_{(2,18)} = 44.79$, $P < 0.001$). The interaction effect of temperature and salinity was statistically significant (ANOVA, $F_{(2,18)} = 40.06$, $P < 0.001$). The cultures at 20℃ and 4.0 ppt had significantly higher C:P ratio than all the other cultures (Tukey's HSD test, $P < 0.05$). The cultures at 20℃ and 1.0 ppt also had significantly higher C:P ratio than all the other cultures (Tukey's HSD test, $P < 0.05$).

3.1.10. Chlorophyll a of Phase 2

Figure 3.12 illustrates that in Phase 2 *P. parvum* had an increasing chlorophyll a concentration with increasing salinity and temperature.

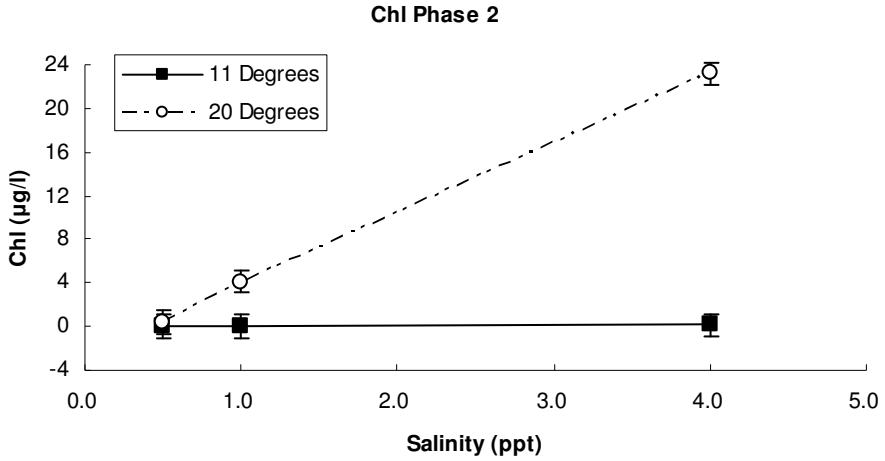


Figure 3.12 Chlorophyll a of Phase 2. Error bars represent standard error based on the mean square error of the ANOVA.

In Phase 2, the experimental effect of temperature was statistically significant (ANOVA, $F_{(1,18)} = 119.51$, $P < 0.001$). The experimental effect of salinity was statistically significant (ANOVA, $F_{(2,18)} = 71.45$, $P < 0.001$). The interaction effect of temperature and salinity was statistically significant (ANOVA, $F_{(2,18)} = 69.64$, $P < 0.001$). The cultures at 20℃ and 4.0 ppt had significantly higher chlorophyll a concentration than all the other cultures (Tukey's HSD test, $P < 0.05$).

3.1.11. Chlorophyll a *Quota of Phase 2*

Chlorophyll *a* quota was obtained by dividing chlorophyll a concentration by end point density. Figure 3.13 showed that *P. parvum* tended to have a decreasing chlorophyll *a* quota with decreasing salinity.

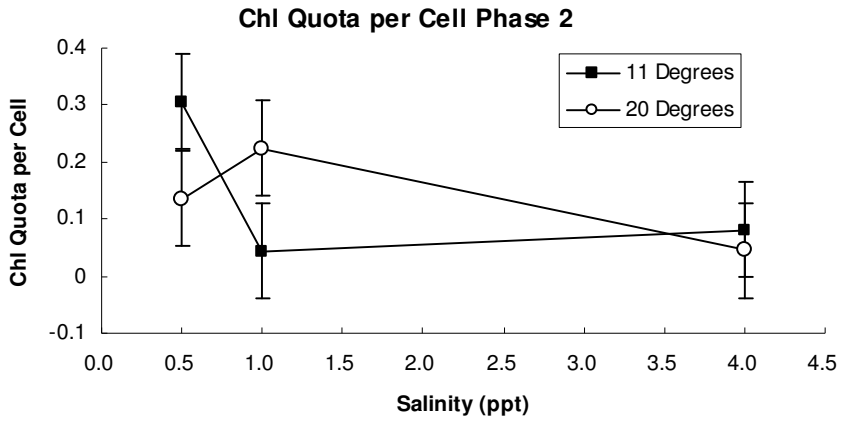


Figure 3.13 Chlorophyll *a* quota of Phase 2. Error bars represent standard error based on the mean square error of the ANOVA.

In Phase 2, the experimental effect of temperature was not statistically significant (ANOVA, $F_{(1,18)} = 0.02$, $P = 0.89$). The experimental effect of salinity was not statistically significant (ANOVA, $F_{(2,18)} = 1.77$, $P = 0.20$). The interaction effect of temperature and salinity was not statistically significant (ANOVA, $F_{(2,18)} = 2.23$, $P = 0.14$).

3.1.12. N:P Ratio of Phase 2

The N:P ratio was obtained by dividing N quota by P quota. Figure 3.14 illustrates that *P. parvum* had an increasing N:P ratio with increasing salinity and temperature.

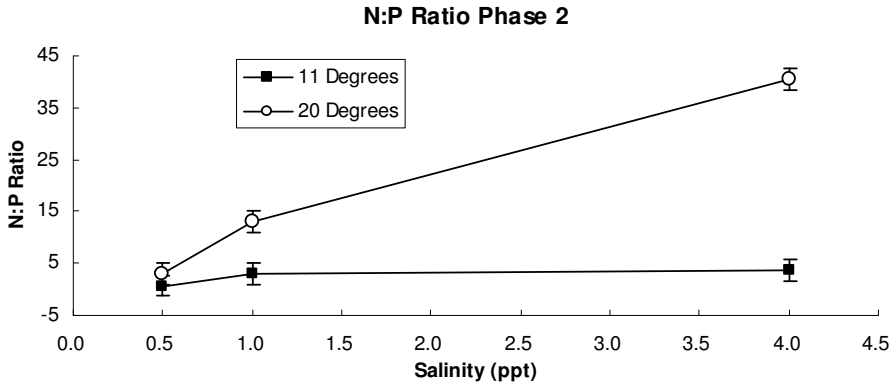


Figure 3.14 N:P ratio of Phase 2. Error bars represent standard error based on the mean square error of the ANOVA.

In Phase 2, the experimental effect of temperature was statistically significant (ANOVA, $F_{(1,18)} = 92.11$, $P < 0.001$). The experimental effect of salinity was statistically significant (ANOVA, $F_{(2,18)} = 50.37$, $P < 0.001$). The interaction effect of temperature and salinity was

statistically significant (ANOVA, $F_{(2,18)} = 38.13$, $P < 0.001$). The cultures at 20℃ and 4.0 ppt had significantly higher N:P than all the other cultures (Tukey's HSD test, $P < 0.05$).

3.2 Phase 1 versus Phase 2 Contrasts

For each type of data Phase 1 was contrasted versus Phase 2 by subtracting Phase 1 data from Phase 2 data, for each unique culture. Then, a paired t-test (Statistica 6.0, StarSoft, Tulsa, Oklahoma, USA)was conducted for Phase 1 vs. Phase 2, by testing the null hypothesis of a zero difference between phases. This results (Table 3.2) show that the differences of exponential growth rate, pH, P quota, and log end point density were significant ($P < 0.05$). Because these results indicate differences between phases for 5 out of 8 responses, we analyzed how the difference varies with experimental treatment in the following sections.

Table 3.2 Paired t-test of Phase 1 vs Phase 2 for exponential growth rate, pH, P Quota, coded lysis, log end point density and percent lysis per cell.

	Mean	Standard Deviation	N	Standard Error	t-value	Degrees of Freedom	P
Exponential Growth Rate (per Day)	-0.054	0.11	24	0.0232	-2.31	23	0.03
Log End Point Density	-1.37	2.23	24	0.4546	-3.00	23	0.006
pH	0.45	0.89	24	0.1816	2.50	23	0.02
P Quota per Cell	2.37×10^{-6}	4.46×10^{-6}	24	9.10×10^{-7}	2.60	23	0.02
Coded Percent Lysis (%)	-3.56	16.88	24	3.4452	-1.03	23	0.3
Percent Lysis per Cell	0.02	0.07	24	0.0149	1.34	23	0.2
C Quota	9.36×10^{-5}	1.77×10^{-4}	24	3.60×10^{-5}	2.60	23	0.02
C:P	-15.33	50.31	24	10.27	-1.49	23	0.2

3.2.1. Exponential Growth Rate

Contrasts of exponential growth rate differed for different temperatures (Figure 3.15). At the lower temperature, *P. parvum* grew more slowly during Phase 2, while at the higher temperature, growth was similar in the two Phases.

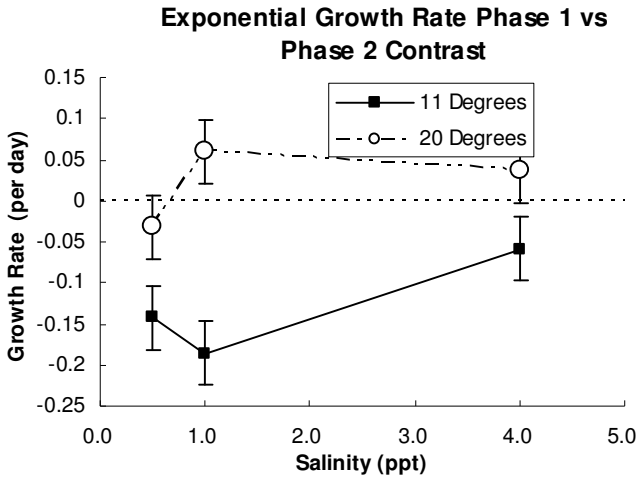


Figure 3.15 Exponential growth rate of Phase 1 vs Phase 2. Error bars represent standard error based on the mean square error of the ANOVA.

The experimental effect of temperature was statistically significant (ANOVA, $F_{(1,18)}$ = 22.32, P < 0.001). The experimental effect of salinity was not statistically significant (ANOVA, $F_{(2,18)}$ = 2.00, P = 0.164). The interaction effect of temperature and salinity was not statistically significant (ANOVA, $F_{(2,18)}$ = 2.27, P = 0.132).

3.2.2. End Point Density

The contrast for end point density at 11 degrees shows that *P. parvum* grow worse during Phase 2 than Phase 1 (Figure 3.16). There was no significant difference at 20 degrees.

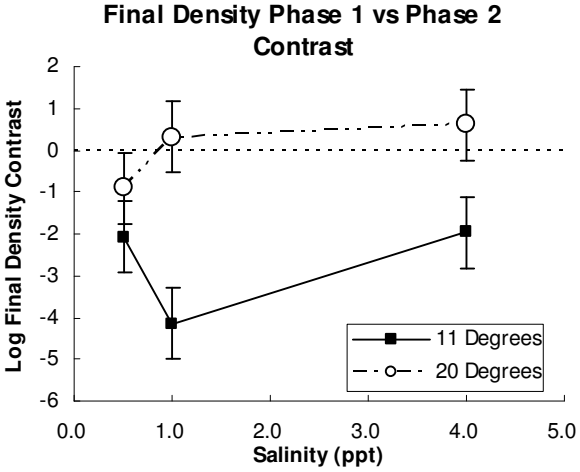


Figure 3.16 Log end point density of Phase 1 vs Phase 2. Error bars represent standard error based on the mean square error of the ANOVA.

The experimental effect of temperature was statistically significant (ANOVA, $F_{(1,18)}$ = 15.53, P < 0.001). The experimental effect of salinity was not statistically significant (ANOVA, $F_{(2,18)}$ = 1.10, P = 0.354). The interaction effect of temperature and salinity was not statistically significant (ANOVA, $F_{(2,18)}$ = 1.85, P = 0.19).

3.2.3. pH Values

The contrast for pH at 20 degrees shows that *P. parvum* had higher pH during Phase 2 than Phase 1 (Figure 3.17). There was no significant difference at 11 degrees.

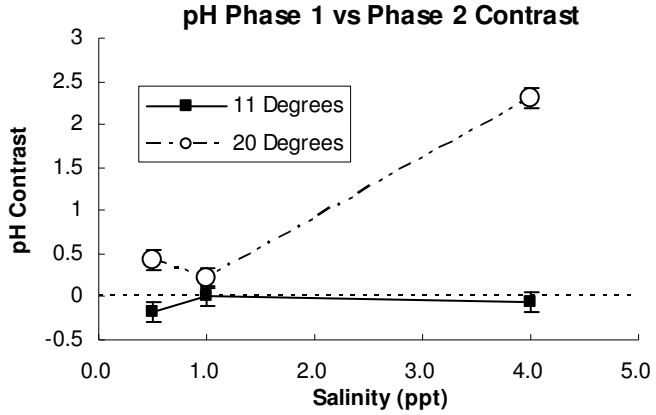


Figure 3.17 pH values of Phase 1 vs Phase 2. Error bars represent standard error based on the mean square error of the ANOVA.

The experimental effect of temperature was statistically significant (ANOVA, $F_{(1,18)}$ = 126.44, P < 0.001). The experimental effect of salinity was statistically significant (ANOVA, $F_{(2,18)}$ = 51.27, P < 0.001). The interaction effect of temperature and salinity was statistically significant (ANOVA, $F_{(2,18)}$ = 49.11, P < 0.001). The cultures at 20℃ and 4.0 ppt had significantly greater difference of pH value between 2 phases than all the other cultures (Tukey's HSD test, P < 0.05).

3.2.4. P Quota

The contrast for P quota per cell at 11 degrees showed that *P. parvum* had higher values during Phase 2 than Phase 1 (Figure 3.18). There was no significant difference at 20 degrees.

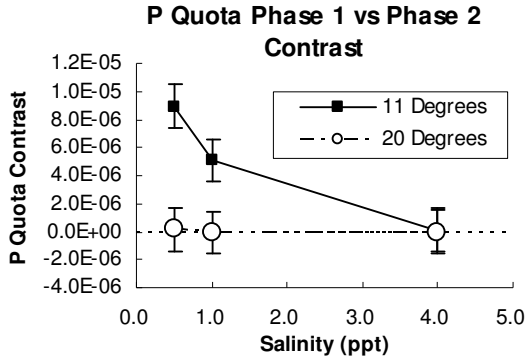


Figure 3.18 P quota of Phase 1 vs Phase 2. Error bars represent standard error based on the mean square error of the ANOVA.

The experimental effect of temperature was statistically significant (ANOVA, $F_{(1,18)}$ = 13.84, P = 0.002). The experimental effect of salinity was statistically significant (ANOVA, $F_{(2,18)}$ = 4.26, P = 0.031). The interaction effect of temperature and salinity was statistically significant (ANOVA, $F_{(2,18)}$ = 4.002, P = 0.036). The cultures at 11℃ and 0.5 ppt had significantly greater difference of P quota between 2 phases than all the other cultures except for cultures at 11℃ and 1.0 ppt (Tukey's HSD test, P < 0.05).

3.2.5. Percent Lysis

Contrast of percent lysis was obtained using original data without transformation since the distribution of contrast data is close to normal. Figure 3.19 illustrated that at 11℃ *P. parvum* are more toxic during Phase 2 than Phase 1; while at 20 ℃, Phase 2 was less toxic.

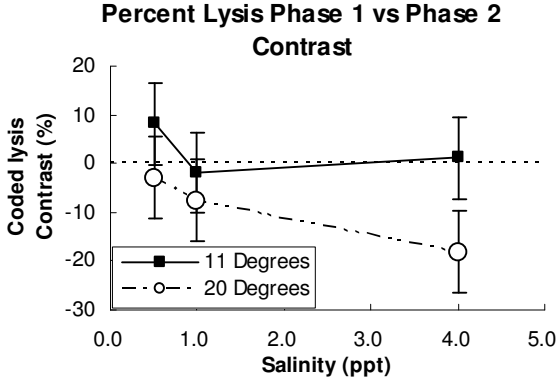


Figure 3.19 Percent lysis of Phase 1 vs Phase 2. Error bars represent standard error based on the mean square error of the ANOVA.

Although overall statistical analysis showed no significant between factors, there were specific treatments that had a large difference. The experimental effect of temperature was statistically insignificant (ANOVA, $F_{(1,18)} = 3.14$, $P = 0.094$). The experimental effect of salinity was not statistically significant (ANOVA, $F_{(2,18)} = 0.92$, $P = 0.416$. The interaction effect of temperature and salinity was not statistically significant (ANOVA, $F_{(2,18)} = 0.33$, $P = 0.72$).

3.2.6. Percent Lysis per Cell

When toxic activity per cell was calculated, the contrast between Phase 1 and Phase 2 indicated no significant difference between the two phases at 20℃. However, the 11℃ contrast showed that Phase 2 had more toxicity per cell than Phase 1(Figure 3.20).

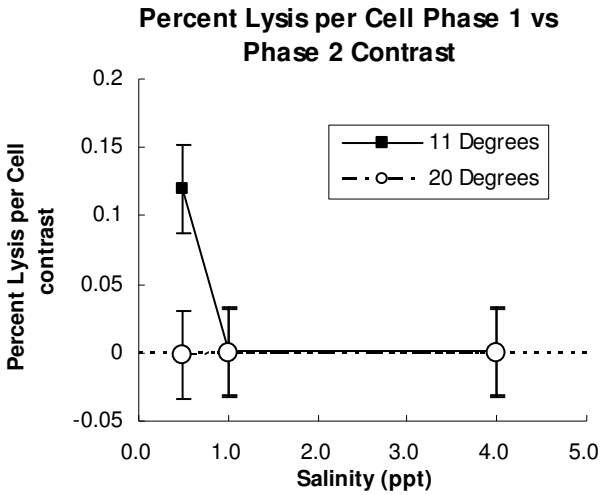


Figure 3.20 Percent lysis per cell of Phase 1 vs Phase 2. Error bars represent standard error based on the mean square error of the ANOVA.

The experimental effect of temperature was not statistically significant (ANOVA, $F_{(1,18)} = 2.53$, $P = 0.13$). The experimental effect of salinity was not statistically significant (ANOVA, $F_{(2,18)} = 2.21$, $P = 0.139$). The interaction effect of temperature and salinity was not statistically significant (ANOVA, $F_{(2,18)} = 2.36$, $P = 0.12$).

3.2.7. C Quota

The contrast for C quota per cell at 11 degrees showed that *P. parvum* had higher value during Phase 2 than Phase 1 (Figure 3.21). There was no significant difference at 20 degrees.

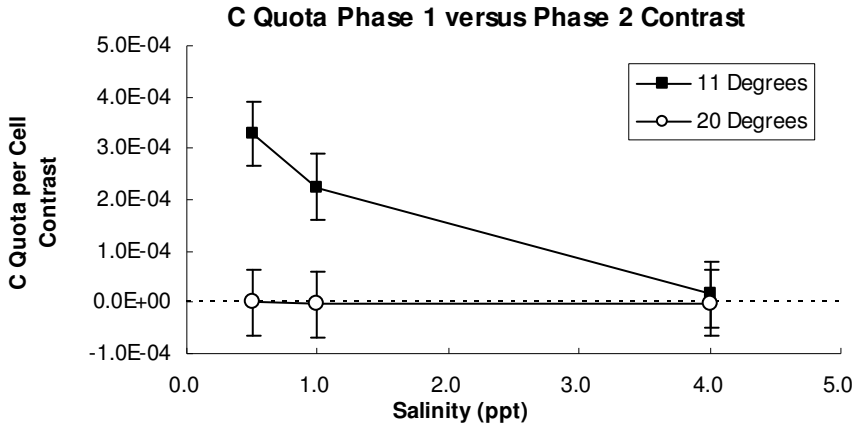


Figure 3.21 C quota per cell of Phase 1 vs Phase 2. Error bars represent standard error based on the mean square error of the ANOVA.

The experimental effect of temperature was statistically significant (ANOVA, $F_{(1,18)}$ = 13.38, P = 0.002). The experimental effect of salinity was not statistically significant (ANOVA, $F_{(2,18)}$ = 3.12, P = 0.07). The interaction effect of temperature and salinity was not statistically significant (ANOVA, $F_{(2,18)}$ = 3.11, P = 0.07).

3.2.8. C:P Ratio

The contrast for C:P ratio at 0.5 ppt showed that *P. parvum* had higher value during Phase 1 than Phase 2 for both temperature (Figure 3.22). There was no significant difference at 1.0 ppt and 4.0 ppt.

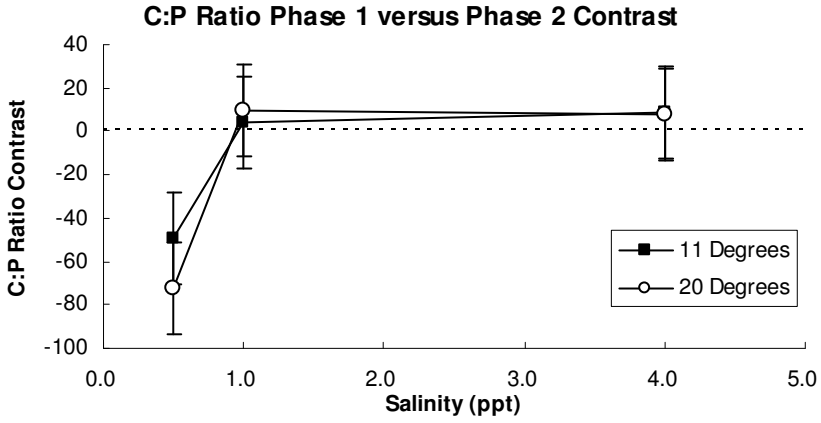


Figure 3.22 C:P ratio of Phase 1 vs Phase 2. Error bars represent standard error based on the mean square error of the ANOVA.

The experimental effect of temperature was not statistically significant (ANOVA, $F_{(1,18)}$ = 0.13, P = 0.73). The experimental effect of salinity was statistically significant (ANOVA, $F_{(2,18)}$ = 6.90, P = 0.006). The interaction effect of temperature and salinity was not statistically significant (ANOVA, $F_{(2,18)}$ = 0.25, P = 0.78).

CHAPTER 4

DISCUSSION AND CONCLUSIONS

This study shows, in general, that *P. parvum* grows more rapidly at higher temperature and higher salinity during both phases of long-term repeated culturing. When the two phases of culturing are compared, *P. parvum* grows more slowly at 11°C during Phase 2, while growth differs little at 20°C. Toxicity differs little between phases. These results show no evidence of acclimation or adaptation during culturing over the length of time examined, and thus rejecting the main hypothesis of this study. These results also contradict earlier suggestions that growth performance of algal cultures improves over 15 – 20 generations of culturing (Brand 1982). Qualitatively, at least, these results support earlier short-term experiments indicating that this Texas strain of *P. parvum* displays slower growth at temperatures < 20 °C and salinities < 2 ppt than it does at higher temperatures and salinities (Baker et al. 2007, 2009). Broadly similar results have been obtained for other strains of *P. parvum* in short-term experiments (Larsen et al. 1993, Larsen and Bryant 1998).

Figure 4.1 shows exponential growth rate compared to predictions of a regression model based on short-term experiments (Baker et al. 2009). At 11 degrees, the regression predicts negative growth rates at salinity < 2 ppt. However, results from this study provided opposite evidence of positive growth at low salinities of 0.5 ppt and 1.0 ppt, for 11 °C. At 20 °C, growth rate from this study agrees with regression predictions at salinities < 2 ppt. At 4.0 ppt, growth rate from this study is lower than regression predictions. This is likely a result of differences in the way exponential growth rate was calculated in this study compared to Baker et al. (2009). In the earlier work, many cultures displayed distinct exponential and stationary phases, so exponential growth rate was calculated only from day 2 to day 9 to exclude stationary phase data. In this study, exponential growth rate was calculated from the entire

incubation period, because few cultures displayed a distinct stationary phase. Under the most stressful growth conditions (temperature of 11 ℃ and salinities of 0.5 and 1.0 ppt), including a longer period of time apparently enabled positive growth to be detected. Therefore it is possible that earlier experiments underestimated the potential growth rate of *P. parvum* populations under these stressful conditions. However, this underestimation was not due to lack of acclimation or adaptation, but possibly due to the short interval (7 days) used to estimate growth rate under stressful conditions.

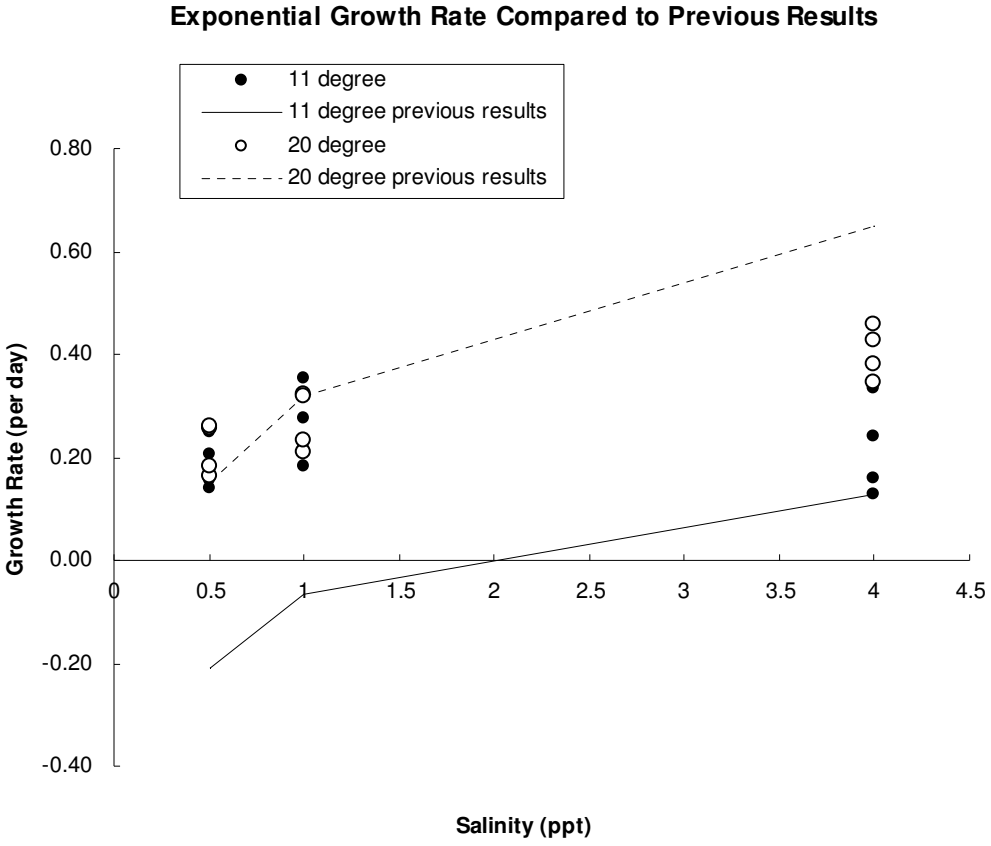


Figure 4.1 Exponential growth rate compared to values predicted from a regression equation based on short-term experiments (Baker et al. 2009).

Lack of adaptation in growth rate (due natural selection), as implied by the results of this study, could be a consequence of the low genetic diversity expected in cultures. Even those cultures that are not truly clonal are initiated from a small number of individuals. However, some

previous work has suggested that even clonal cultures can display some degree of acclimation or adaptation (Brand 1982). A limitation of this study is that natural populations likely have more genetic variability than the one cultured strain examined here. Thus they would be expected to be able to adapt more rapidly to stressful conditions than a cultured population. However, the populations of *P. parvum* in prevalent in Texas may not have a lot of genetic variability, due to what appears to be the recent invasion of this region. Most sampled populations in one study were genetically similar to one particular group of *P. parvum* in the old world (Lutz-Carillo et al. 2010).

In conclusion, this study produced no evidence that *P. parvum* improves its ability to grow under low temperature and salinity through acclimation or adaptation, over the time period of culturing used here. Such acclimation and adaptation were excluded by the relatively short duration of previous experiments that suggest *P. parvum* grows poorly under low temperature and salinity. Had such acclimation or adaptation been observed, it would have provided a potential explanation for why *P. parvum* can grow to bloom levels during winter in Texas, when temperature and salinity are below optimal, and growth rates are expected to be low. Instead, this study suggests that previous experiments might have underestimated growth rates of *P. parvum* at low temperature and salinity due to a different methodological limitation, the choice of a relatively short time period over which to estimate exponential growth rate.

REFERENCES

Baker, J.W., J.P. Grover, B.W. Brooks, F. Ureña-Boeck, D.L. Roelke, R. Errera, and R.L. Kiesling., 2007. Growth and Toxicity of *Prymnesim parvum* (Haptophyta) as a Function of Salinity, Light, and Temperature, Journal of the Phycological Society of America 43:219–227.

Baker, J. W., J.P. Grover, R. Ramachandrannair, C. Black, T.W. Valenti, Jr., B.W. Brooks, and D.L. Roelke, 2009. Growth at the Edge of the Niche: An Experimental Study of the Harmful Alga *Prymnesium parvum*, Limnology 54(5).

Brand, L.E, 1982. Genetic Variability and Spatial Patterns of Genetic Differentiation in the Reproductive Rates of the Marine *coccolithophores Emiliania huxleyi* and *Gephyrocapsa oceanica*. Limnol. Oceanogr., 27(2):236-245.

Brooks, B.W., S.V. James, T.W. Valenti, Jr., F. Urena-Boeck, C. Serrano, J.P. Berninger, L. Schwierzke, L.D. Mydlarz, J.P. Grover, and D.L. Roelke. 2010. Comparative Toxicity of *Prymnesium parvum* in Inland Waters. Journal of the Phycological Society of America 46(1):45-62.

Granéli, E. and P.S. Salomon. Factors Influencing Allelpathy and Toxicity in *Prymnesium parvum*. Journal of the American Water Resources Association 2010. 46(1):108-120.

Glibert, P.M., D. Anderson, P. Gentien, E. Granéli, and K.G. Sellner, 2005. The Global, Complex Phenomena of Harmful Algal Blooms. Oceanography 18(2):130-141.

Granéli, E. and J.T. Turner, 2006. An Introduction to Harmful Algae. In: Ecology of Harmful Algae, E. Granéli, and J.T. Turner (Editors). Ecological Studies Series Vol. 189: Springer Verlag, Berlin, Heidelberg, Germany, pp. 3-7.

Grover, J.P., J.W. Baker , F. Ureña-Boeck, B.W. Brooks, R.M. Errera, D. L. Roelke, and R. L. Kiesling. 2007. Laboratory Tests of Ammonium and Barley Straw Extract as Agents to

Suppress Abundance of the Harmful Alga *Prymnesium Parvum* and its Toxicity to Fish. Water Res. 41: 2503–2512.

Grover, J.P., J.W.Baker, D.L.Roelke, and B.W. Brooks. 2010. Current Status of Mathematical Models for Population Dynamics of *Prymnesium Parvum* in a Texas Reservoir, Journal of the American Water Resources Association 46(1):92-107.

Kester, D. R., Duedell, I. W., Connors, D. N. & Pytkowicz, R. M.1967. Preparation of Artificial Seawater. Limnol. Oceanogr.12:176–9.

Kleinbaum, D. G., L. L. Kupper, K. E. Muller, and A. Nizam. 1998. Applied Regression Analysis and Other Multivariable Methods. Duxbury Press, Pacific Grove, California, USA.

Larsen, A. & Bryant, S. 1998. Growth Rate and Toxicity of *Prymnesium parvum* and *Prymnesium patelliferum* (Haptophyta) in Response to Changes in Salinity, light and Temperature. *Sarsia* 83:409–18.

Larsen, A., Eikrem, W. & Paasche, E. 1993. Growth and Toxicity in *Prymnesium patelliferum* (Prymnesiophyceae) Isolated from Norwegian Waters. *Can. J. Bot*. 71:1357–62.

Lutz-Carillo, D. J., G. M. Southard, and L. T. Fries. 2010. Global genetic relationships among isolates of golden algae (*Prymnesium parvum*). Journal of the American Water Resources Association, 46(1):24-32.

Roelke, D.L., L. Schwierzke, B.W. Brooks, J.P. Grover, R.M. Errera, T.W. Valenti, Jr., and J.L. Pinckney. 2010. Factors Influencing *Prymnesium parvum* Population Dynamics During Bloom Initiation: Results from In-lake Mesocosm Experiments. Journal of the American Water Resources Association, 46(1):76-91.

Schwierzke, L., D.L. Roelke, B.W. Brooks, J.P. Grover, T.W. Valenti, Jr., M. Lahousse, C.J. Miller, and J.L. Pinckney. 2010. *Prymnesium parvum* Population Dynamics During Bloom Development: A Role Assessment of Grazers and Virus. Journal of the American Water Resources Association, 46(1):63-75

Strickland, J.D.H., and T.R. Parsons. 1972. A practical handbook of seawater analysis, 2nd. ed. Bulletin of the Fisheries Research Board of Canada 167.

Texas Parks &Wildlife. 2003. *Prymnesium parvum* Workshop Report for 2003. http://www.tpwd.state.tx.us/landwater/water/environconcerns/hab/ga/workshop/

Van Dolah, F.M., 2000. Marine Algal Toxins: Origins, Health Effects, and Their Increased Occurrence. Environmental Health Perspectives 108(S1):133-141.

Watson, Sean. 2001. Literature Review of the Microalga *P. parvum* and its Associated Toxicity. Texas Parks and Wildlife Department. PWD RP T3200-1158 (8/01).

Welschmeyer, N.A. 1994. Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and phaeopigments. Limnology and Oceanography 39: 1985-1992.

BIOGRAPHICAL INFORMATION

Xianzi He was born in Suzhou, China. She received her Bachelor of Science in Ecology from China Agricultural University, Beijing, China. Her research interests broadly are Aquatic Ecology, Algal Ecology, Biodiversity, Environmental Health, and Environmental Engineering. So far, her course work and research work has been focused on the listed areas. In the Master's study and this thesis, she conducted experiments to investigate ecology of *Prymnesium parvum* under stressful conditions.

After graduating with her Master's, she wants to study further on aquatic ecology and environmental engineering. And after gained more scientific engineering experience, she would like to pursue for a doctoral degree in the future.